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DOI: https://doi.org/10.1007/s11259-005-3301-1

Veterinary Research Communications, 29 (2005) 647–659

Evaluation of an Electrolyte Analyser for Measurement of Concentrations of Ionized Calcium and Magnesium in Cats

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Unterer, S., Gerber, B., Glaus, T.M., Hässig, M. and Reusch, C.E., 2005. Evaluation of an electrolyte analyser for measurement of concentrations of ionized calcium and magnesium in cats. *Veterinary Research Communications*, **29(8)**, 647–659

ABSTRACT

The goal of this study was to evaluate the Nova CRT 8 electrolyte analyser for determination of concentrations of ionized calcium (Ca_i) and magnesium (Mg_i) in cats, to determine the effects of sample handling and storage and to establish reference ranges. The precision and analytical accuracy of the Nova CRT 8 analyser were good. The concentrations of Ca_i and Mg_i were significantly lower in aerobically handled serum samples than in those handled anaerobically. The concentrations of Ca_i and Mg_i differed significantly among whole blood, plasma and serum. In anaerobically handled serum, the concentration of Ca_i was stable for 8 h at 22°C, for 5 days at 4°C and for 1 week at –20°C. The concentration of Mg_i was stable for 4 h at 22°C but for less than 24 h at 4°C and for less than 1 week at –20°C. In serum from 36 cats, the reference ranges were 1.20–1.35 mmol/L for Ca_i and 0.47–0.59 mmol/L for Mg_i. The Nova CRT 8 electrolyte analyser is suitable for determination of Ca_i and Mg_i concentrations in cats. Anaerobically handled serum samples are recommended and, stored at room temperature, they yield accurate results when analysed within 4 h.

Keywords: Nova CRT 8 electrolyte analyser, ionized calcium, ionized magnesium, cats

Abbreviations: Ca_i, ionized calcium, Mg_i, ionized magnesium

INTRODUCTION

Calcium and magnesium are two essential cations that play an important role in numerous metabolic and cellular functions. Recent studies have investigated the role of these electrolytes in various feline disorders including idiopathic hypercalcaemia (Midkiff *et al.*, 2000), calcium oxalate urolithiasis associated with hypercalcaemia (McClain *et al.*, 1999; Savary *et al.*, 2000) and clinically relevant hypocalcaemia associated with pancreatitis (Kimmel *et al.*, 2001), urethral obstruction (Drobatz and Hughes, 1997) and lymphoma involving large granular lymphocytes (Wellman *et al.*, 1992). The role of magnesium in intensive care management has also received particular attention in cats (Norris *et al.*, 1999; Wooldridge and Gregory, 1999; Toll *et al.*, 2002). In critically ill humans (Broner *et al.*, 1990; Rubeiz *et al.*, 1993), dogs (Martin *et al.*, 1999) and cats (Toll *et al.*, 2002), abnormalities in magnesium concentration have been correlated with high rates of morbidity and mortality.

In blood, both electrolytes may be protein-bound, free of complexed with other substances. Only the free ionized fraction if biologically active and is maintained in a narrow concentration range by various interacting feedback loops (Rosol *et al*., 2000). The serum concentrations of free calcium and magnesium may be affected by total serum protein concentration, serum pH, presence of carrier proteins and individual protein-binding affinity and, thus, do not always correlate with the respective total concentrations (Chew *et al*., 1989; Deniz and Mischke, 1995; Kulpmann and Gerlach, 1996; Rosol *et al*., 2000; Jutkowitz *et al*., 2002). Therefore, it would be advantageous to directly measure the concentration of ionized calcium (Ca_i) and magnesium (Mg_i) os that clinically relevant abnormalities are more reliably detected. Worldwide, there are only a few types of electrolyte analysers available for the measurement of the concentrations of Ca_i and Mg_i, using ion-selective electrodes (Cao *et al*., 2001). These analysers have been used in human medicine for a number of years (Bowers *et al*., 1986; Thode *et al*., 1989; Zoppi *et al*., 1996; Markova *et al*., 1997; Hoshimo *et al*., 2001) and to some extent in veterinary medicine (Deniz and Mischke, 1995; Drobatz and Hughes, 1997; Mann *et al*., 1998; Wooldridge and Gregory, 1999; Kimmel *et al*., 2001; Bolliger *et al*., 2002). To the authors' knowledge, detailed results of the evaluation of electrolyte analysers for the determination of Ca_i and Mg_i in cats have not been published.

The goals of this study were to evaluate the Nova CRT 8 analyser for determination of Ca_i and Mg_i in healthy cats, to investigate various factors affecting measurements and to establish analyser-specific reference ranges for cats.

MATERIALS AND METHODS

Analyser

The Nova CRT 8 electrolyte analyser is equipped with ion-selective electrodes and simultaneously measures haematocrit, pH and the concentrations of Na^+, K^+, Mg^{2+} and Ca^{2+} in whole blood, plasma and serum. The analyser provides results for Ca_i (measurement range 0.1–5.00 mmol/L) and Mg_i (measurement range 0.1–2.5 mmol/L). It also provides calculated results for these electrolytes normalized to pH 7.4. The equation used for this calculation is:

$$\log [\text{electrolyte}]_{\text{pH7.4}} = \log [\text{electrolyte}]x - 0.24 \times (7.4 - X)$$

where X is the measured pH of the sample. In addition, the electrical signal from the Mg^{2+}-selective electrode is adjusted for the signal from the Ca^{2+} electrode by an algorithm that uses the selectivity constant K_{MgCa}. The analyser performs a two-point calibration with two Ca^{2+} and Mg^{2+} aqueous solutions. A sample volume of 180 µl is required and the measurement cycle is 55 s (operating instructions, Nova Biomedical, Rödermark, Germany).

Case material

Forty adult cats owned by employees of the Faculty of Veterinary Medicine, University of Zurich, were used for this study. The cats were considered healthy on the basis of history and the results of physical examination, a complete blood count and biochemical profile. The cats belonged to four different breeds and ranged in age from 0.5 to 14 years (mean 5.5 years). There were 10 intact female, 8 spayed female, 10 intact male and 12 castrated male cats. Of the 40 cats, 20 were randomly selected to form three subgroups for study of the effects of sample handling and storage conditions on electrolyte concentrations: samples from 6 cats were used to investigate the effect of air; samples from 7 cats were used for comparison of different types of blood specimens and to determine serum sample stability at 22°C; and samples of another 7 cats were used to study serum sample stability at 4°C and –20°C. Of the 40 cats, only those above 1 year of age, i.e. 36 cats, were used to establish reference ranges for Ca_i and Mg_i concentrations.

Evaluation of the analyser

Precision. For the determination of precision in a measurement series, two standard samples from the manufacturer (Nova Chemistry Control, Nova Biomedical, Waltham, MA, USA), one with a low (Ca_i, 0.59–0.83 mmol/L; Mg_i, 0.26–0.42 mmol/L and one with a high concentration (Ca_i, 1.50–1.90 mmol/L; Mg_i, 1.32–1.68 mmol/L) of Ca_i and Mg_i, and a feline serum sample with mid-range (Ca_i, 1.25 mmol/L; Mg_i, 0.43 mmol/L) concentrations of Ca_i and Mg_i were measured ten times within 10 min. For day-to-day precision, the manufacturer's two standard samples with low and high concentrations of Ca_i and Mg_i were measured every 24 h for 10 consecutive days. Samples were stored anaerobically at 4°C.

Analytical accuracy. For the determination of analytical accuracy, linearity of serial dilution results and of the concentrations of the electrolytes by prepared solutions was assessed. Highly concentrated aqueous solutions of calcium (4.84 mmol/L) and magnesium (2.49 mmol/L) were prepared from crystalline calcium chloride (calcium chloride 2-hydrate crystalline, Merk, Zurich, Switzerland) and crystalline magnesium chloride (magnesium chloride 6-hydrate crystalline, Merk), respectively. These stock solutions were diluted with isotonic saline to make 50%, 25%, 12.5% and 6.25% solutions. For each solution, the concentration of Ca_i and Mg_i was measured with the analyser and compared to the calculated values. Ca_i and Mg_i aqueous solutions (Ca_i = 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 mmol/L; Mg_i = 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 mmol/L) were prepared. Different Ca_i and Mg_i concentrations were achieved by adding different amounts of either 0.1 mol/L calcium (Calcium Ions Standard Solution, 0.1 mol/L, $Cacl_2{\cdot}2H_2O$, Fluka Chemie, Buchs, Switzerland) or 0.1 mol/L magnesium (Magnesium Ion Standard Solution, 0.1 mol/L, $MgCl_2{\cdot}6H_2O$, Fluka) ion standard solutions to 10 ml of isotonic saline. Direct measurements were compared to actual electrolyte concentrations.

Sample handling and stability

Effect of air. To determine how the exposure of specimens to air affects measurements, blood was collected from the jugular vein of 6 cats and immediately transferred into three polypropylene tubes without anticoagulant (5 ml tubes with push cap, Sarstedt AG, Sevelen, Switzerland) such that 100%, 50% and 25% of the repetitive tube was filled. In tubes that were completely filled (100%), inclusion of visible air bubbles was carefully avoided to achieve completely anaerobic conditions. Fifteen minutes later, the samples were centrifuged at 7500*g* for 2–5 min, followed immediately by measurement of concentrations of Ca_i and Mg_i. The results of the tubes with the different air contents were compared.

Comparison of Ca_i and Mg_i concentrations in whole blood, plasma and serum. For determination of Ca_i and Mg_i concentrations in whole blood, samples were collected from 7 cats into heparin-coated syringes (3 ml syringe containing $\sim$50 units of lyophilized lithium and zinc heparin, Ownes-BriGam Medical Company, Morganton, NC, USA), and measurements were made immediately from the tip of the syringe. Thereafter, the remaining blood was placed in an Eppendorf serum tube (1.5 ml Eppendorf microtubes with attached 'safety cap', polypropylene, Sarstedt AG) for harvesting plasma, avoiding the inclusion of visible air bubbles. Within 5 min, a second blood sample was collected from each cat into an Eppendorf serum tube without anticoagulant for harvesting serum. All tubes were allowed to sit for 15 min, after which they were centrifuged at 7500g for 2–5 min, before Ca_i and Mg_i were measured in plasma and serum.

Stability of samples stored anaerobically at different temperatures. Aliquots of 0.5 ml of serum were placed in airtight tubes so that the tube was completely filled (0.5 ml microtubes with screw cap, polypropylene, Sarstedt AG). Serum samples from 7 cats were stored at 22°C. Serum samples from 7 different cats were stored at 4°C and at –20°C. For all three temperatures, the first measurement was performed immediately after centrifugation of the blood. For samples stored at 22°C, additional measurements were made 1, 2, 3, 4, 8, 24 and 48 h later. For samples stored at 4°C, additional measurements were made every 24 h for 5 days. For samples stored at –20°C, additional measurements were made 1, 3, 11 and 26 weeks later. The frozen samples were thawed for 30 min at 22°C before processing. The measurements obtained after storage were compared to the values of the first measurement. A sample was considered stable up to the time at which the measured values were first significantly different from the values measured immediately after sampling.

Reference values

Sample collection and handling. A blood sample was collected from the jugular vein of all 36 cats and placed into an Eppendorf serum tube (1.5 ml Eppendorf microtubes with attached 'safety cap', polypropylene, Sarstedt AG). The tubes were filled

completely, excluding any visible air bubbles, and closed tightly. After 10–15 min, the tubes were centrifuged at 7500g for 2–5 min and the concentrations of Ca_i and Mg_i were immediately determined.

Statistical methods

Data were compiled and statistical analysis was performed using a commercial computer program (SPSS 11.0 for Windows, SPSS Inc, Chicago, IL, USA). Data were subjected to the K-S-test for normality of the distribution. Precision was calculated using coefficients of variation. Linearity was assessed using regression analysis and correlation coefficients. Student's *t*-test for paired samples was used for the comparison of the means of results from tests of stability and handling influences. Sample stability was analysed using Student's *t*-test for paired samples. Reference ranges were defined as the range from the 5th to the 95th centile. Unless otherwise stated, values are reported as mean $\pm$ SD, and the unit of concentration is mmol/L. Differences were considered statistically significant at $p \leqslant 0.05$.

RESULTS

Evaluation of the analyser

Precision. The coefficients of variation for precision in a measurement series for the different concentrations of Ca_i and Mg_i ranged from 0.45% to 0.87%. Those for day-to-day precision ranged from 1.15% to 2.29% (Table I).

TABLE I
Coefficients of variation of measurements of Ca_i and Mg_i in samples with different levels of Ca_i and Mg_i

		Coefficient of variation (%)	
	Concentration range (mmol/L)	Ten measurements within 10 min	Ten measurements on 10 consecutive days
Ca_i low	0.59–0.83	0.87	2.21
Ca_i high	1.50–1.90	0.60	1.15
Ca_i midrange (feline serum)	1.23–1.26	0.79	
Mg_i low	0.26–0.42	0.79	2.29
Mg_i high	1.32–1.68	0.45	1.96
Mg_i midrange (feline serum)	0.43–0.44	0.73	

Analytical accuracy. Linearity measurements for both Ca_i and Mg_i showed very good agreement between the calculated and measured concentrations for the entire measurement range of the analyser (Ca_i, $R = 1.000$; Mg_i, $R = 0.999$).

Recovery of Ca_i ranged from 95.4% to 105.0% (mean 98.0%) and recovery of Mg_i ranged from 88.0% to 100.0% (mean 92.4%). There was a linear relationship between the measured and actual concentrations of Ca_i and Mg_i. The regression curves for Ca_i and Mg_i, respectively, were $y = 0.05+0.94x$ and $y = 0.03+0.89x$, and the correlation coefficients were $R = 1.00$ in both (Figure 1). The slopes of the regression lines were significantly different from 0 in both and the constant was significantly different from 0 in Ca_i.

Sample handling and stability

Effect of air. The concentrations of Ca_i in tubes filled to 25% capacity (1.26 ± 0.04 mmol/L) and 50% capacity (1.28 ± 0.04 mmol/L) were significantly lower than the concentrations in tubes that were completely filled (1.32 ± 0.04 mmol/L). The same was true for Mg_i, with concentrations of 0.52 ± 0.06 mmol/L in tubes filled to 25%, 0.53 ± 0.06 mmol/L in tubes filled to 50%, and 0.55 ± 0.06 mmol/L in completely filled tubes (Figure 2). The pH measured in 25% filled tubes (7.57 ± 0.04) was significantly higher than in 50% filled (7.51 ± 0.03) or completely filled (7.42 ± 0.03) tubes. The difference in pH between 50% filled tubes and completely filled tubes was also significant.

Comparison of Ca_i and Mg_i in whole blood, plasma and serum. The concentration of Ca_i was significantly lower in plasma (1.30 ± 0.04 mmol/L) than in whole blood (1.35 ± 0.04 mmol/L) and serum (1.33 ± 0.04 mmol/L). The concentration of Mg_i was significantly lower in serum (0.60 ± 0.04 mmol/L) than in whole blood (0.66 ± 0.11 mmol/L) and plasma 0.67 ± 0.07 mmol/L) (Figure 3). The pH at the time of the measurement was significantly higher in plasma (7.47 ± 0.05) compared to whole blood (7.39 ± 0.03) or serum (7.39 ± 0.07).

Stability of samples stored anaerobically at different temperatures. The concentration of Ca_i was stable for 8 h at 22°C, for 5 days at 4°C and for 1 week at –20°C. The concentration of Mg_i was stable for 4 h at 22°C. After 24 h at 4°C and after 1 week at –20°C, the concentration of Mg_i was significantly higher than before storage (Table II). At 22°C, the pH of the samples increased by 0.01 units after 4 h. Although the difference was minimal, the pH was significantly different from the initial pH at times 4 h, 8 h and 24 h, respectively. No significant change in pH was measured at 4°C storage temperature, while at –20°C the pH was significantly increased after 1 week and thereafter.

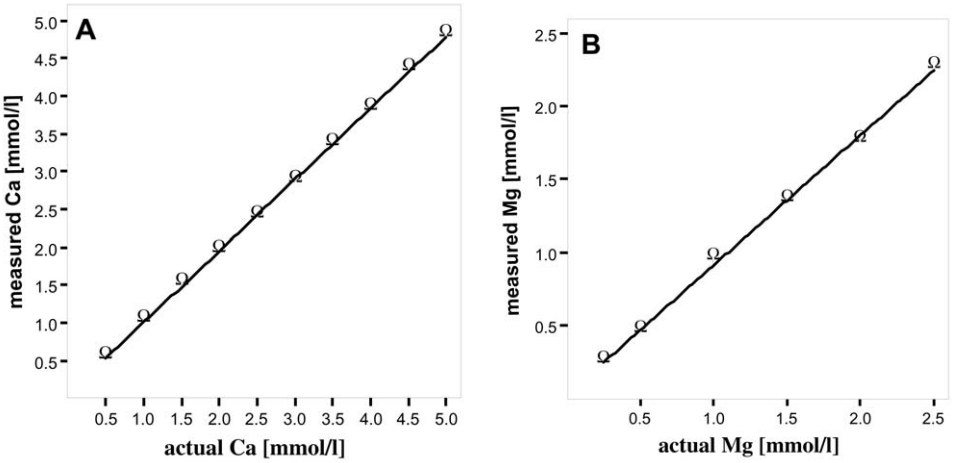

Figure 1. The linearity of measurements of different ionized calcium (A) and magnesium (B) aqueous solutions obtained with the Nova CRT 8 analyser. Direct measurements of the electrolyte concentrations were compared with actual amounts of Ca_i or Mg_i in aqueous solutions. The equations and correlations for the linear regressions for Ca_i were $y = 0.05 + 0.94x$, $R = 1.00$ and for Mg_i were $y = 0.03 + 0.89x$, $R = 1.00$

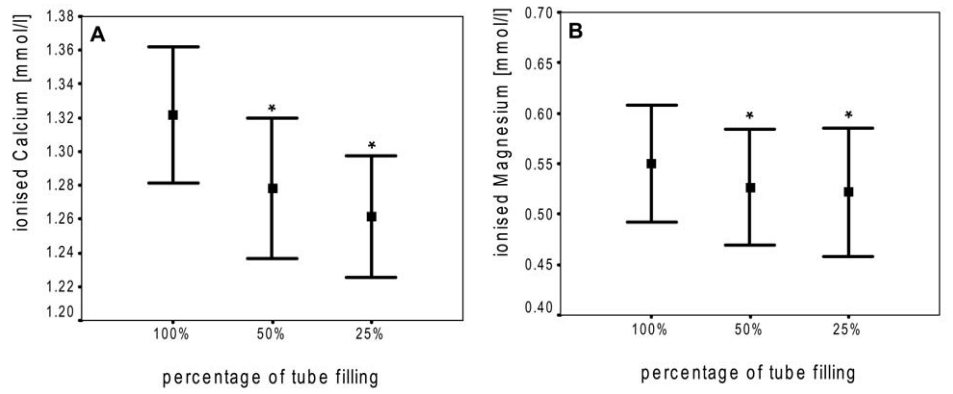

Figure 2. The effect of air on the ionized calcium (A) and magnesium (B). The serum from a blood sample from each of 6 cats was divided into three tubes such that one tube was completely filled, one was filled to 50% capacity and the third was filled to 25% capacity. Twenty minutes later, the concentrations of ionized calcium and magnesium were determined and the results for each of the three tubes from one cat were compared. An asterix represents measurements that differ significantly from that of the tube that was completely filled with serum

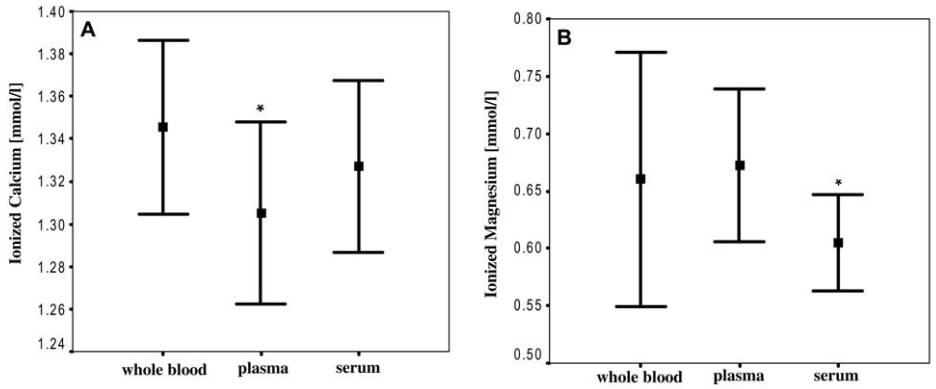

Figure 3. Comparison of the concentrations of ionized calcium (A) and magnesium (B) in whole blood, plasma and serum from 7 cats. An asterix represents measurements that were significantly lower than those of the other two sample types

Reference values

The reference ranges using anaerobically handled serum were 1.20–1.35 mmol/L for Ca_i and 0.47–0.59 mmol/L for Mg_i.

DISCUSSION

This study demonstrated that the Nova CRT 8 analyser is suitable for the measurement of Ca_i and Mg_i in cats. However, our results, as well as those of other studies (Landt *et al.*, 1994; Schenk *et al.*, 1995; Rosol *et al.*, 2000), have shown that special handling and storage of samples is critical for accurate results. In samples that are not handled anaerobically, carbon dioxide escapes, resulting in an increase in sample pH. Because there are fewer hydrogen ions available in these samples, the hydrogen-binding sites on proteins become occupied by ionized electrolytes, resulting in decreased concentrations of Ca_i and Mg_i (Szenci *et al.*, 1991; Schenk *et al.*, 1995; Rosol *et al.*, 2000). Our results demonstrate the importance of strict anaerobic handling of samples; serum samples stored in tubes that were only half full had significantly lower Ca_i and Mg_i concentrations 20 min after preparation than had anaerobically handled samples, while the pH increased significantly.

Ionized calcium and magnesium can be measured in whole blood, plasma or serum. Harvesting of whole blood and plasma requires an anticoagulant, which may affect the free electrolyte fraction in different ways. Anticoagulants may bind with ionized electrolytes and thereby reduce their concentration, or electrolytes of the anticoagulant may interfere with the electrode function or displace bound electrolytes

TABLE II
The effect of different storage temperatures on the stability of ionized calcium and magnesium in anaerobically handled serum from 7 healthy cats (values are means ± SD)

Time	Ionized calcium (mmol/L)	Ionized magnesium (mmol/L)	pH measured at time of analysis
	at 22° C	at 22° C	
0	1.271 ± 0.058	0.437 ± 0.082	7.41 ± 0.05
1 h	1.270 ± 0.055	0.434 ± 0.088	7.41 ± 0.05
2 h	1.274 ± 0.049	0.437 ± 0.085	7.41 ± 0.05
4 h	1.271 ± 0.048	0.440 ± 0.087	7.42 ± 0.05*
8 h	1.273 ± 0.063	**0.447 ± 0.087***	7.42 ± 0.05*
24 h	**1.251 ± 0.051***	0.476 ± 0.091*	7.42 ± 0.05*
48 h	1.276 ± 0.055	0.461 ± 0.084*	7.42 ± 0.05
	(at 4°C)	(at 4°C)	
0	1.299 ± 0.047	0.540 ± 0.052	7.42 ± 0.04
24 h	1.313 ± 0.043	**0.563 ± 0.054***	7.41 ± 0.03
48 h	1.301 ± 0.043	0.561 ± 0.056*	7.41 ± 0.04
72 h	1.299 ± 0.039	0.570 ± 0.065*	7.41 ± 0.04
96 h	1.294 ± 0.042	0.544 ± 0.058	7.41 ± 0.05
120 h	1.311 ± 0.053	0.551 ± 0.053*	7.39 ± 0.06
	(at –20°C)	(at –20°C)	
0	1.299 ± 0.047	0.540 ± 0.052	7.41 ± 0.04
1 week	1.306 ± 0.052	**0.601 ± 0.068***	7.44 ± 0.04*
3 weeks	**1.233 ± 0.052***	0.547 ± 0.055	7.49 ± 0.05*
11 weeks	1.210 ± 0.021*	0.547 ± 0.061	7.54 ± 0.05*
26 weeks	1.097 ± 0.065*	0.541 ± 0.076	7.72 ± 0.09*

*Significant difference from time 0, $p \leqslant 0.05$

Values of ionized calcium and ionized magnesium in bold type represent the first values that differed significantly from the values at times 0

from serum proteins and, thus, result in increased values (Landt *et al.*, 1994; Swanson, 1994; Lyon *et al.*, 1995; Ritter *et al.*, 1996). In the present study, the concentration of Ca_i was significantly lower in plasma than in serum, which was thought to be due to the formation of complexes of heparin and Ca_i in plasma. The plasma concentration of Ca_i was also significantly lower than that of whole blood, although both specimens contained the same anticoagulant. This difference may have been attributable to a delay in the measurements in plasma. Measurements in whole blood were made immediately after collection and from the tip of the syringe, whereas those in plasma were performed a few minutes later. Possibly, this delay allowed for a larger portion of

the heparin from the coated syringe to dissolve and to form complexes with the Ca_i. Measured pH in plasma was higher than in whole blood or serum, and this might have been the only reason for the increased Ca_i in plasma compared to whole blood or serum; however, the difference in pH was not high enough to influence Mg_i. The concentration of Mg_i was significantly lower in serum than in whole blood or plasma. Presumably, this was due to zinc ions in the anticoagulant used (Altura *et al.*, 1994; Lyon *et al.*, 1995; Ritter *et al.*, 1996). It was speculated that coagulation in serum would increase Mg_i owing to release of Mg_i from blood cells (Dimai *et al.*, 2000). The low serum-Mg_i in our study indicates that this effect, if present, is not large enough to counteract others. Our results indicate that measurements differ among whole blood, plasma and serum and that the results of one cannot be substituted for another. We therefore recommend the use of serum for measurement of Ca_i and Mg_i because an anticoagulant is not required and serum samples remain stable for a longer period (Rosol *et al.*, 2000).

Mg_i values obtained in serum samples of six cats used to compare influence of different types of blood were slightly above the reference range. This may be explained by a significantly elevated Mg_i level in two cats (0.65 and 0.68 mmol/L), which were used for this part of the evaluation but were excluded from the reference range study because of their young ages.

The results of this study indicate that, for in-house analysis, serum does not need to be refrigerated because the concentration of Ca_i remains stable at room temperature for 8 h and the concentration of Mg_i for 4 h. Refrigeration (4°C) is advised for serum samples if determination of Ca_i concentration is not feasible within 8 h. In contrast, the concentration of Mg_i was significantly decreased after 24 h of storage at 4°C and after 1 week at –20°C. Thus, reliable Mg_i measurements in serum samples stored at room temperature can only be expected within 4 h of collection. For a more accurate determination of the stability of Mg_i in samples stored at 4°C or –20°C, measurements performed at shorter time intervals would be necessary. Storage at room temperature and at –20°C led to increased pH and this might have led to a significant decrease in Ca_i. In contrast, Mg_i increased with increasing storage time. Increasing Mg_i with increasing pH was also found in another study (Altura *et al.*, 1994). In that study an increase in pH minimally increased Mg_i in the opposite direction of Ca_i in an aqueous solution, while freezing and thawing of plasma leading to an increased pH minimally affected Mg_i. Our results also showed no significant changes in Mg_i after 3, 11 and 26 weeks of frozen storage, indicating that the influence of pH was neutralized by some other influences.

Reference ranges should be determined according to the type of specimen (plasma, serum or whole blood), species and age of animal, and type of analyser used. Reference ranges determined in humans (Hristova *et al.*, 1995; Rehak *et al.*, 1996; Cecco *et al.*, 1997; Huijgen *et al.*, 1999) using different electrolyte analysers have shown that there are marked analyser-specific differences. A reference range for Ca_i in cats (1.0–1.4 mmol/L), measured with a Nova CRT 8 analyser, was also reported by Bolliger and colleagues (2002), but no details of how it was established were given. Their reference range is wider than ours; the same is true for those reported by Rosol and Capen (1997) or Rosol and colleagues (2000) (1.07–1.47 and 1.1–1.4 mmol/L, respectively).

The latter two reports also give no details on how these ranges were established. Our reference range for Ca_i is close to that established by Dzenis and Mischke (1995), measured with a 634 Ca^{2+}/pH Analyzer (Ciba Corning Diagnostics GmbH, Fernwald, FRG) from plasma (1.15–1.37 mmol/L). According to our results, the measured concentration of Ca_i in plasma is lower than in serum. In the former study, this could have been counterbalanced by the age of the animals used. Ca_i was higher in younger animals and almost half of the cats were 1 year of age or younger in contrast to our study where only values of cats older than 1 year of age were used to calculate the reference range. Wooldridge and Gregory (1999) report the range for Ca_i and Mg_i from only 10 cats. In both ranges the upper end is lower than our reference range (Ca_i, 1.19–1.27 mmol/L; Mg_i, 0.45–0.53 mmol/L). Nothing is known about the cats used. Reference ranges for Mg_i established in another study from 12 cats were similar to ours (Norris et al., 1999: Mg_i reference range 0.44–0.58 mmol/L).

In addition to the actual measurements, the Nova CRT 8 electrolyte analyser provides values that are corrected for pH 7.4. The pH correction was not used in the present study because it has not been validated for cats. Changes in pH occurring *in vivo*, which may influence the ionized electrolyte concentration of a patient, are ignored by this correction. The actual measurements from an anaerobically handled sample are considered to be the most accurate (Rosol *et al.*, 2000).

The coefficient of variation (CV) for measurements in series given by the manufacturer (operating instructions, Nova Biomedical, Rödermark, Germany) is 2% for both Ca_i and Mg_i and CV for precision from day to day is 3% for Ca_i and 4% for Mg_i. We obtained CV results that were below these values. CV values for measurements in series were 0.60–0.87% for Ca_i and 0.45–0.79% for Mg_i. CV values for precision from day to day were 1.15–2.21% for Ca_i and 1.96–2.29% for Mg_i.

In conclusion, the Nova CRT 8 analyser is suitable for clinic use. The concentration of Ca_i in anaerobically handled, cooled serum samples remains stable long enough for shipment to a laboratory. In contrast, the concentration of Mg_i is less stable, even at lower temperatures, and should be measured within 4 h of collection for accurate results. The reference ranges determined here for Ca_i and Mg_i apply only to the Nova CRT 8 analyser.

ACKNOWLEDGEMENT

This work is part of the dissertation of Stefan Unterer, Clinic of Small Animal Internal Medicine, University of Zurich, Switzerland.

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(Accepted: 8 July 2004)